2004

Precipitation of SKY Kits and QC

Section of Cancer Genomics, Genetics Branch, NCI
National Institutes of Health

Reagents

Ethanol, absolute
Chromosome paints: product of SKY labeling PCR (see protocol)
Dextran Sulfate (50%)
Intergen, Cat. S4030
Formamide, deionized (pH 7)
Ambion, Cat. 9342
Human Cot – 1 DNA
Gibco BRL, Cat. 15279 – 011
Salmon sperm DNA
Sigma Molecular Biology, Cat. D – 7657
Sodium Acetate (3M)
SSC, 20X

Preparation

Master Mix

Dextran sulfate, 50%	40 ml
20X SSC, pH 7	10 ml
Sterile dH_2O	50 ml

Vortex solution and place on a shaking platform overnight to insure proper mixing.
Aliquot, and store at –20˚C

Procedure

1. For each SKY probe, combine in a 1.5 ml microcentrifuge tube, 4 μl aliquots of each SKY-labeling PCR product. This will give a total of 57 PCR reactions x 4 μl = 228 μl total volume.

2. To this add 20 μl of human Cot-1 DNA and 1 μl of salmon sperm DNA, bringing the total volume to 249 μl.

3. Add 1/10 the total volume sodium acetate.

4. Add 2.5 times the total volume of ethanol (–10 ˚C), in this case 800 µl.

5. Vortex and store overnight at –20 ˚C or at –80 ˚C for 30 min.

6. Centrifuge precipitated DNA at 13,000 rpm for 30 min at 4 ˚C.

7. Carefully pour off the supernatant and spin in speed vac with the tube uncapped for 10 min (medium heat) until pellet is dry.

8. Add 5 µl of deionized formamide to each tube and incubate at 37^0C for 30 min while shaking in a thermomixer.

9. After the pellet has fully dissolved, add 5 µl of Master Mix, vortex and spin briefly. After this step the probe is now ready to either denature or freeze at -20^0C.

Notes

1. To guarantee that the probe will dissolve successfully in the formamide, the precipitated probe must be thoroughly dried and no ethanol remains in the tube.

2. In order to minimize photo-bleaching of the probe, try to minimize exposure of the probe to ambient light.